(54) Title: HIGH PERFORMANCE CELL CULTURE BIOREACTOR AND METHOD
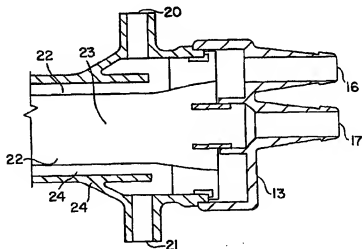
(57) Abstract

     A high performance hollow fiber bioreactor having concentric hollow fiber bundles (22, 23) is disclosed. The central hollow fiber bundle (23) supplies culture medium while the outer hollow fiber bundle (22) supplies oxygen needed for cell culture. Methods for use of the high performance bioreactor include, for example, to expand stem cells ex vivo are described.

## HIGH PERFORMANCE CELL
## CULTURE BIOREACTOR AND METHOD

### FIELD OF THE INVENTION

This invention relates to hollow fiber bioreactors. More particularly, this invention relates to a novel high performance bioreactor and to a method for its utilization.

### BACKGROUND OF THE INVENTION

Prior art bioreactors conventionally perfuse nutrient media through a single type of hollow fiber. The various disadvantages of such bioreactors include heterogeneous cell mass, difficult procurement of representative cell growth samples, and poor performance due to inefficient oxygenation and an inability to control oxygen levels. Moreover, microenvironmental factors such as pH cannot be effectively controlled. Mixed or co-culture of cells is not possible.

### DEFINITIONS

In the specification and claims, the following terms have the meaning set forth below.

HPBr--High Performance Bioreactor.

Extracapillary Space (ECS)--The space within the bioreactor cartridge which is not occupied by the hollow fibers.

Intracapillary Space (ICS)--The totality of the space defined by the lumens of the hollow fibers in a bioreactor.

OXY-1--A hollow fiber oxygenator available from UniSyn Technologies, Inc., 14272 Franklin Avenue, Suite 106, Tustin, California 92680. The OXY-1 bioreactor is provided with 0.2 $\mu$m pore size polyethylene hollow fibers woven to form a mat. The active surface area for gas exchange is 1ft$^2$.

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BR110--A small hollow fiber bioreactor
commercially available from UniSyn Technologies,
Inc.  The BR110 hollow fibers are cellulosic, with
10kD molecular weight cutoff (MWc).  The active
surface area for cell growth is $1.5ft^2$.

Micro Mouse BR110--A sterile disposable
bioreactor device equipped with a BR110 bioreactor
with a length of silicone tubing in the ICS loop for
gas exchange available from UniSyn Technologies, Inc.

Cell-Pharm 1000--A hollow fiber bioreactor
instrument commercially available from UniSyn
Technologies, Inc.  The Cell-Pharm 1000 system
includes a controller, pH sensor, gas mixture control
capability, media reservoir, heater and peristaltic
pump and pinch valves.

MAb--Monoclonal antibody.

Mitsubishi Oxygenator Fibers--0.2 $\mu$m pore size
polypropylene single strands of hollow fibers from
Mitsubishi, Japan.

Glucose Utilization Rate (GUR)--The rate at which
glucose is consumed from the nutrient media in a
batch feed system (in units of mass per unit time,
e.g. gm/day) on the day prior to the current sample.
This is derived as follows:

$$GUR = F(G_f - G) + V(G_1 - G), \quad G = (G_1 + G_2)/2$$

where $G_f$ is the glucose concentration in IC media
feeding (i.e. -4.1g/L); $G_1$, the glucose concentration
(g/L) in IC recirculating media on the day prior to
the current sample; $G_2$, the glucose concentration
(g/L) in IC recirculating media in the current
sample, F, the IC media feeding rate (L/day), and V,
the volume of IC media in reservoir (L).

Lactate Production Rate (LPR)--The rate at which
lactate (or rather lactic acid) is produced
anaerobically while glucose is consumed.  Therefore,
one molecule of glucose produces two molecules of

lactic acid without oxygen involved in the reaction.
Lactate production rate (LPR), g/day is defined as:

$$LPR = [F(L-L_f) + V(L-L_1)] \times 0.09, \quad L = (L_1+L_2)/2$$

where $L_f$ is the lactate concentration in
intracapillary (IC) media (i.e. 0.1 mM); $L_1$, the
lactate concentration (mM) in IC recirculating media
on the date prior to the current sample; $L_2$, the
lactate concentration (mM) in IC recirculating media.

## SUMMARY OF THE INVENTION

It has been discovered that inefficient
oxygenation is a focal constraint upon the efficiency
of hollow fiber cell culture devices.  The invention
provides a novel bioreactor which achieves greatly
enhanced nutrient media oxygenation as compared with
prior art devices.

Pursuant to the invention, nutrient media is
supplied through an inner passage concentric to and
hence surrounded by an outer bundle of oxygenator
hollow fibers.  The inner media passage is provided
by a single, media porous tube or by an inner bundle
of media supply hollow fibers.  The exterior surface
of the outer or oxygenator fiber bundle is spaced
from the inner wall of the bioreactor housing to
facilitate the mixing of cellular material and the
sampling or harvesting of product cells.

The high performance bioreactors of the invention
is particularly suited for the growth of highly
oxygen sensitive cells, such as insect cells used in

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the production of biomolecules for commercial
purposes.

## CONSTRUCTION OF THE
## HIGH PERFORMANCE BIOREACTOR

This portion of this specification describes, by
reference to Figures 1 to 10, the best mode presently
known to the inventors for the construction of a
bioreactor which embodies the invention.

### Description of Figures 1 to 10

Figure 1 is a schematic illustration of a
bioreactor of the invention.

Figure 2 is a cross-section of one of two
substantially identical ends of a bioreactor as shown
by Figure 1.

Figure 3 depicts the hollow fiber housing and the
critical components which enable efficient and
scaleable fabrication of high performance bioreactor.

Figure 3A is an end view of the hollow fiber
housing of Figure 3.

Figure 4 shows the Figure 3 assembly in the mold
with potting compound.

Figure 5 illustrates the potted ends of the
cartridge after removal from the mold, with the two
cut marks for removal of excess adhesive and fiber
indicated.  Note that the mask component is
sacrificial and is removed with excess adhesive.  The
hollow fiber guide remains imbedded in the adhesive
as a permanent part of the end seal in the hollow
fiber housing.

Figure 6 depicts the cut end of the bioreactor
cartridge.

Figure 7 shows the final assembly including the
dual port header, which provides a means to supply
media to the inner bundle of fibers and oxygen
containing gas to the outer concentric ring of fibers.

Figure 8 is a detailed scale drawing of the fiber guide. This component provides the means for both organizing the two types of fibers, and enables sealing of the central fiber bundle to an independent port from that of the outer bundle of fibers.

Figure 9 is a detailed scale drawing of the sacrificial mask which fits over the portion of the fiber guide to provide a clean surface for sealing the central fiber bundle port.

Figure 10 is a detailed scale drawing of the dual port header for supplying media to the central bundle of fibers and oxygenated gas to the outer fibers independently.

Referring to Figure 1, the bioreactor 10 includes a housing 11 having substantially identical headers 12 and 13 at its input and output ends. Headers 12 and 13 have ports 14, 15, 16 and 17 for the introduction and withdrawal of oxygen containing gas and media.

Figure 2 illustrates an outer oxygenator fiber bundle 22 concentric with an inner media supply fiber bundle 23. The exterior periphery of the oxygenator bundle 22 is separated by space 24 from the inner wall of the housing 11. Header ports 14 and 16 accommodate the introduction of oxygen containing gas into and withdrawal of oxygen containing gas from the oxygenator bundle 22. Header ports 15 and 17 accommodate the passage of nutrient media into and the removal of nutrient media from the nutrient media supply bundle 22.

Product cells or proteins are sampled and harvested from space 24 through ports 18, 19, 20 or 21. Inoculation cells may be introduced through ports 18, 19, 20 or 21.

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A preferred method of fabricating a bioreactor embodying the invention includes six major steps now described by reference to Figures 3 to 10.

1. Prepare 4 3/4 inch length fiber bundles from fiber tows with the requisite number of strands. Wrap the end of each bundle with tape so that fibers are held securely.

2. Assemble jacket (10) with the fiber guide (10a) and mask (10b) as shown in Figure 3.

3. Preheat and prespin cellulose fiber bundles for 20 minutes at 1800 RPM and 52°C to remove excess glycerine processing aid. Carefully insert cellulose fibers through the central hole in the fiber guides at each end of the jacket assembly shown in Figure 3A. Similarly, put three bundles of oxygenator fibers through the three lateral holes in the fiber guide and ensure that they are loosely packed. Further drying of the bundles is done at 65°C for 14-18 hours.

4. Place the warm jacket/bundle assembly in a potting mold to encase the free ends of the bundles as shown in Figure 4. This assembly is placed in a centrifuge with premixed polyurethane resin in an applicator. Spin the entire assembly at 1500 RPM for 50-60 minutes at 38-45°C.

The resin enters the jacket assembly (10) through either ports 18 and 20, or 19 and 21.

5. Cut the warm cured resin at the potted ends of the jacket/bundle assembly along the two "cut" lines shown in Figure 5. Remove the cut pieces along with the mask (10b shown in Figure 3), to expose the clean surface of the fiber guide (10a in Figure 3). This surface participates in sealing the header.

6.  The header is installed after applying
polyurethane resin to the clean surface exposed by
removing the mask, and to the outer rim of the
jacket (10).  Curing occurs at room temperature over
a 24 hour period.

### Media Supply Fiber Types

Hollow fibers represent a specific group of
tubular articles that are within the scope of this
invention.  Therefore, any porous tube or fiber that
is capable of being integrated into a recirculating
(ICS) loop, while enabling perfusion of nutrient
media to cells growing on the outside, is included in
this invention.  The following are examples of tubes
or fibers preferred for use in this invention:

        (i)  Hollow fibers and tubes with dialysis,
    ultrafiltration and microfiltration properties,
    i.e., (a) molecular weight cutoff (MWc) ranging
    from 1 kD to 1,000 kD; and (b) pore size ranging
    from 0.01 $\mu$m to 5.0 $\mu$m,
        The most preferred range is from 10 kD MWc
    and about up to 1 $\mu$m pore size.
        (ii)  Materials of construction include
    polymers, graphite, ceramics (including porous
    glass fiber) and metals (e.g., stainless steel).
    Typically, polymers are required to have good
    physical properties (e.g., tensile strength, melt
    temperature and glass transition temperature).
    These include cellulose, polyethylene,
    polypropylene, polysulfone (and other engineering
    thermoplastics), polymethyl methacrylate,
    polyacrylonitrile, various polymer blends and the
    like.
        Specifically preferred media supply fibers are
from cellulose polymers, have a MWc of
10-1,000 kD and a pore size of 0.01-1.0 $\mu$m.

### Oxygenator Fiber Types

Hollow fibers are the only tubular configuration
selected for oxygenation in this invention.  These
hollow fibers can be porous or non-porous.  Porous
hollow fibers for oxygenation are typically
hydrophobic (like those made from polyethylene,
polypropylene, polytetrafluoroethylene (Teflon) and
the like).  Examples of non-porous hollow fibers
include (but are not restricted to) silicone, and
silicone copolymer capillary tubing.  They typically
have high gas permeability through the solid wall
structure.

Porous hydrophobic hollow fibers are most
effective for gas exchange when the pore size is in
the range of 0.01 to 0.5 $\mu$m.  Optimally, pore size
should be in the range of 0.1 to 0.2 $\mu$m.

Specifically preferred oxygenator fibers are
formed from polypropylene and a pore size of 0.15 $\mu$m.

### Discussion of Operation

The HPBr is equipped with the means for
recirculating nutrient media via the lumen (or ICS)
of a hollow fiber bundle or other tubular structures
centrally located in the bioreactor jacket.  These
hollow fibers or tubes are selected for their
permeability properties, and in some instances for
their surface properties (e.g., hydrophilicity or
hydrophobicity).  Media profused through the porous
wall of these structures bath cells that have been
inoculated in the ECS.

$O_2/CO_2$ gas mixtures are supplied to the HPBr
through the lumen tube or a bundle of hollow fibers
that are situated concentric to the centrally placed
hollow fibers or tubes.  The gas mix flows counter
current to the flow of media.  One important
operating parameter is the gas pressure in the

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oxygenator fibers.  The optimal pressure is a
function of the number of strands of fibers used, and
the pressure of the perfusing media in the ECS.  For
example, a bioreactor embodiment described in the
examples that had 180 Mitsubishi polypropylene fibers
showed a bubble point of >2.5-3 psi.  For a HPBr with
540 oxygenator fibers, the bubble point was closer to
about 2 psi.  As cell mass increases and cells adhere
directly to the fibers, the bubble point will tend to
increase.

     Table 1 illustrates the relationship between the
number of oxygenator and media fibers for two types
of HPBr's described in Example II (i.e., Type I
and II).  Two additional HPBr configurations
(Type III and IV), and an OXY-1/BR110 combination are
included for comparison.  The figure demonstrates
that as the ratio of oxygenator fiber surface area to
media fiber surface area increases, oxygenation
efficiency also increases at the expense of the
extracapillary space available for cell culture.

     HPBr Type IV represents a more extreme case
covered by this invention, where the annular cell
culture space is within the range for a BR110
(conventional) bioreactor.  The ECS of 11.8 mL is
within the range expected from about 70% (maximum)
fiber packing density for a BR110 or OXY-1.  (The
maximum space in the potted jacket with absolutely no
fiber present is about 22 mL.)  For the embodiment
described in Example II, the oxygenator/media fiber
surface area ratio range is between 0.03 to 5.5.  The
preferred range is between 0.7 and 2.6.  As this data
shows, the invention may be regarded as an oxygenator
device within which cells are cultured, and media
supply is provided by a relatively few porous hollow
fibers or a large porous tube, e.g., a graphite tube.

TABLE 1

Relationship Between Oxygenator and
Media Supply Fibers in HPBr

| | OXY-1/ BR110 Combination | High Performance Bioreactors | | | |
|---|---|---|---|---|---|
| | | Type I | Type II | Type III | Type IV* |
| Oxygenator Fiber S.A. (ft$^2$) | 1.0 | 0.1 | 0.3 | 0.5 | 1.1 |
| Media Fiber S.A. (ft$^2$) | 1.5 | 0.2 | 0.2 | 0.2 | 1.2 |
| Oxy/Media Fiber Ratio | 0.7 | 0.5 | 1.5 | 2.5 | 5.5 |
| Extracapillary Space (mL) | n/a | 19.5 | 18.0 | 16.4 | 11.8 |

*Note:  Based on 2000 media fibers at a maximum packing
packing density (i.e., ~ 70%).

    The gas composition is important in defining the
efficiency of oxygenation as well as the pH in the
micro-environment for cell growth created in the
ECS.  Illustrative data showing the effect on pH is
presented in Example II.  This invention permits more
accurate monitoring and control of pH in the optimal
range required than had been possible previously.
    Provision is made in the bioreactor for an
annular space between the outer most oxygenator
fibers and the inner wall of the jacket.  This space
is a specially designed part of the ECS for
containing cell mass.  This culture space facilitates
mixing when the bioreactor is (optimally)
periodically rotated to-and-fro (by 120 degrees).
Further, it facilitates sampling and harvesting of
products (particularly intact viable cells, e.g., for
cellular therapy applications).  This space also
enables microcarriers for anchorage dependent cells
to be contained in the HPBr.  A specific instance

where this is critical is in the culture of
hematopoietic stem cells for cellular therapy
applications.  Stem cells are grown better in the
presence of anchorage dependent stromal cells
(including endothelial cells).  However, stem cells
are anchorage independent.  Therefore, stromal cells
immobilized in or on microcarriers can be co-cultured
with stem cells.  The micro-environment thus created
will facilitate the hitherto unrealized goal of long
term ex-vivo expansion of stem cells.  High yields
and viability for cells from this cell culture space
are achievable.

     Finally, expensive growth factors and cytokines
that are vital to the growth and differentiation of
various cell types can be applied in relatively small
quantities to the ECS to further tailor the
micro-environment.

     Important and unique features of the invention,
both separately and in combination, include: a
relatively high ratio of gas to media supply fibers
in a single jacket; control of oxygenation and pH by
gas composition, pressure and flow rate; relatively
low fiber packing density to provide an
extracapillary space for growing large cell mass and
containing microcarriers; porous tubes or hollow
fibers are employed to supply media and porous or
non-porous hollow fibers are concentrically arranged
to supply oxygenated gases.

     Two different types of hollow fibers are used,
that are made from different materials.  This is
represented by the examples in this invention.

     One type of fiber (e.g., microporous
polypropylene), is utilized where media perfusion and
gas exchange in the respective bundles are achieved
and controlled by regulating pressures.  In

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applications where anchorage independent cells are
being cultured at very high densities, hydrophobic
fiber may be preferred so that cells grow primarily
in suspension and not on the fibers.

     Media supply may be provided by a single porous
tube or by a bundle of hollow fibers.

     Coatings are applied to hollow fiber or tube
surfaces to influence cell attachment (either
positively or negatively).  For example, cellular
matrix molecules found in connective tissue between
cells in-vivo coated onto bioreactor fibers
facilitates adhesion of anchorage dependent cells
like endothelial cells and Chinese hampster (CHO)
cells.  Other molecules containing the active amino
acid sequence arginine-glycine-aspartic acid, called
"RGD" found in matrix molecules also provide this
benefit.  Cellular matrix molecules coated onto HPBr
surfaces facilitate a microenvironment conducive to
efficient gene transfer into hematopoietic stem cells.

     Additional surface area for anchorage dependent
cells is achieved by introducing microcarriers to the
culture space in the ECS.  Co-culturing of different
cell types to gain synergistic benefit is permitted
(e.g., stem cells and stroma).

     Addition of oxygen carriers (particularly
hemoglobin and perflourochemical emulsions) to the
ECS (or ICS), greatly enhances the ability to
accurately control oxygen delivery to the cells and
the rapid growth of cell mass.

                         EXAMPLE I

     Two 15 day cell culture experiments were
conducted with 3C11 hybridoma cell line, that secrets
$IgG_1$ monoclonal antibody in a UniSyn Cell Pharm 1000
pilot scale bioreactor system.  In run 1, three
UniSyn BR110 bioreactor cartridges (1.5 $ft^2$ cellulose

hollow fibers) were equipped with a UniSyn OXY-1
oxygenator (1 ft$^2$, 0.2 $\mu$m pore size polyethylene
oxygenator fibers) for oxygen mass transfer.  In
run 2, three high performance bioreactor (HPBr)
equivalently sized cartridges (#1, #2, and #3) were
fitted to evaluate the capacities of HPBr in 3C11
cell culture.  These HPBr's all had 450 cellulose
hollow fibers (with 10 kD M.W. cut-off) but #1 and #2
had different amounts of oxygenation fibers (in the
form of a mat, as used in OXY-1); and, #3, 504
polyethylene oxygenation fibers.

Figure 11 is a schematic of the flow path for
three BR110 cartridges with one OXY-1 cartridge used
for run 1.  Media recirculation rate was established
by setting the RPM of the media recirculation
peristaltic pump.  The mixed gas containing $CO_2$ and
air was pumped through the oxygenator counter current
to media recirculating in the ICS loop of the
bioreactors by using the gas peristaltic pump.
Calibrating pH and temperature probes were performed
according to the operator's manual.  To control the
pH in the range of 7.0 to 7.4 in ICS media, the
control unit of the Cell Pharm 1000 adjusted the $CO_2$
percentage in the mixed gas automatically.  Also,
this control unit maintained the constant temperature
at 37°C.  Figure 12 is a schematic of the flow path
for the three HPBr cartridges in run 2.  No change
has been made for ICS media flow path relative to
run 2.  However, a modified gas flow path was
designed.  This involved connecting the gas mixture
supply to both the three HPBr's and the media
reservoir.  It should be noted that this crude
modification of the existing Cell Pharm 1000 did not
provide optimal control of gas flow and back
pressure.  Connecting the gas supply with the ICS

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recirculation media reservoir facilitated pH control
by the Cell Pharm 1000 system controller.

    The entire flow path including all tubing,
filters, probes, and reservoirs, renew, and waste
bottles were autoclaved at 120°C for thirty minutes
on the liquid cycle.  Final assembly included
mounting the bioreactor cartridge into the Cell Pharm
1000 System.  Contamination check and system flush
were set to run for 24 hours to ensure sterility,
removal of glycerine (processing aid for cellulose
fibers), and allow for system stability.

    Media conditions:

        (i)  ICS--5% fetal bovine serum (FBS) + 1%
    penicillin/streptomycin + 2% glutamine in 1000 mL
    DMEM.

        (ii)  ECS--20% FBS + 1%
    penicillin/streptomycin + 2% glutamine in 1000 mL
    DMEM.

    Each bioreactor cartridge, including the BR110
and HPBr, were inoculated with the same $3 \times 10^8$ cells
(over 90% viability) into the ECS by using two
sterile, 10 mL syringes with attached 18 gauge
needles.  One syringe contained 5 mL of ECS media and
the cells.  The second syringe was empty and used to
collect the media displaced during the inoculation.

    Product harvest was accomplished in a manner
similar to the inoculation procedure.  Harvesting was
typically done three times a week and 10 mL/each, and
the standard operating procedure for this Cell Pharm
1000 System was followed for maintaining cell culture
daily.

    Process parameters in the cell culture systems
were set up to monitor glucose uptake, lactate
production, $NH_3$ production, MAb production and
perfusion rate of ICS media.  Since one cannot

directly estimate the cell mass present in the hollow
fiber bioreactor at any given time, the MAb
production may be regarded as a means for measuring
the effect of oxygen mass transfer within the two
types of bioreactors in run 1 and 2.  MAb
concentration in the harvest was analyzed by using
radial immunodiffusion assay, glucose, lactate, and
$NH_3$ sampled from the ICS of the bioreactor were
analyzed by using a Kodak Ektachem Analyzer.  The
perfusion rate was determined by measuring the media
level of renew bottles.  Initial perfusion rate was
started at 240 mL/day and was increased step by step
and maximized up to 1000 ml/day until the end of the
runs according to either the glucose concentration
(when it fell as low as 1.5 g/L) or lactate
concentration (when it reached as high as 20 mM).

    Cumulative MAb production in each HPBr cartridge
(run 2) for 3C11 cells, are compared to that in each
BR110 (run 1) in Figure 13.  The ratio of lactate
production rate to glucose consumption rate was
developed as an indicator to evaluate the effect of
oxygen mass transfer.  This ratio was significantly
lower in Run 2 (shown in Figure 4) compared to
Run 1.  These results suggest that the oxygenator
fibers in HPBr cartridges help to reduce the
resistance of oxygen mass transfer to the grow
cells.  This was especially true for the HPBr
cartridge with the Mitsubishi oxygenator fibers that
gave higher MAb production levels than those in the
other two HPBr's which have polyethylene oxygenator
fibers.  It was assumed that the relatively high
dissolved oxygen concentration in the ECS lets cells
grow more efficiently.

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## EXAMPLE II

A 25 day cell culture experiment (run 1) was
conducted with 3C11 hybridoma cell line, that secrets
$IgG_1$ monoclonal antibody.  A test system was designed
to evaluate the performance of three HPBr cartridges
under controlled conditions.  As a reference, a
UniSyn Micro Mouse BR110 small scale bioreactor with
a UniSyn OXY-1 oxygenator was employed in run 2.
Run 3 consisted of a UniSyn Micro Mouse BR110 small
scale bioreactor with a silicone tubing for oxygen
mass transfer by diffusion.

Run 1 used the test system that was designed to
operate HPBr's with the media flow path and the gas
flow rate and pressure manually controlled (see
Figure 14).  A peristaltic pump assembly was used
with three pump heads to provide the same media
recirculation flow through each HPBr at a flow rate
of 100 mL/min.  Temperature in the ECS of each HPBr
was controlled at 37˚C by immersing the three media
reservoirs in a thermostatically controlled
recirculating water bath.  The ICS media pH was
controlled in the range from 7.00 - 7.30 by aerating
the space above the media in the reservoir with $CO_2$
in 75% to 85% air.  This gas mix was also used to
supply gas to the three HPBr cartridges with a
constant flow rate of 38 mL (std(/min and back
pressure of 1.5 psi.

The three HPBr cartridges each had 450 cellulose
hollow fibers for supplying media to the cells by
perfusion.  Both HPBr #1 and #2 had 180 Mitsubishi
oxygenator fibers.  The number of Mitsubishi
oxygenator fibers in HPBr #3 was three times higher
than that in #1 and #2.  For run 1, the contamination
check and system flush were preformed according to

the instructions found in the Micro Mouse operating
manual.

    Run 2 was assembled in an oven for maintaining a
constant temperature (37°).  Premixed air and $CO_2$ was
passed through the OXY-1 oxygenator at a rate of
about 70 mL(std)/min counter current to the direction
of the loop recirculating media in the ICS of the
BR110 bioreactor.  To control pH within the range
from 7.0 to 7.4, the ratio of $CO_2$ to air was adjusted
by using valves on two gas flow meters.

    Run 3 was mounted in a $CO_2$ incubator for
maintaining a constant temperature (37°C) and
constant pH (from 7.0 to 7.2) in the IC media was
established by setting the ratio of 7% $CO_2$ to 93% air
in incubator.

    Media conditions:
        (i)  ICS--5% FBS + 1% penicillin/
    streptomycin + 2% glutamine in 1000 mL DMEM.
        (ii)  ECS--20% FBS + 1% penicillin/
    streptomycin + 2% glutamine in 1000 mL DMEM.

    $3 \times 10^8$ cells (91% viability) were inoculated in
the ECS by using two sterile, 10 mL syringes with 18
gauge needles.  One syringe contained 5 mL of ES
media and cells.  The second syringe was empty and
used to collect the media displaced during the
inoculation.  Both run 2 and 3 were inoculated with 4
$\times 10^8$ cells (95% viability) in the ECS using the
method illustrated above.  The standard operating
procedure for run 2 and 3 was followed for
maintaining cell culture daily.  For run 1, daily
adjustment of back pressure and gas flow rate,
sampling from each ICS media and harvesting from ECS
of each cartridge were carried out.

Process parameters in the cell culture systems were set up to monitor glucose uptake, lactate and $NH_3$ production and MAb production. Cumulative MAb production was monitored to determine the effect of different oxygen mass transfer methods exemplified in runs 1-3.

ICS media for the bioreactors was replaced with fresh media when either the glucose concentration fell below 2.0 g/L or lactate concentration was higher than 20 mM. For run 2 and run 3, harvesting of MAb from the ECS of the bioreactor was done three times weekly and a volume of 10 mL/each (as defined by the Micro Mouse protocol). MAb production was analyzed by using radial immunodiffusion assay, and glucose, lactate, $NH_3$ concentration in harvesting of the ECS of HPBr's were also analyzed. The difference between the levels for these cell metabolites found in the ICS and the ECS of HPBr were noted.

Figure 15 shows the cumulative $IgG_1$ produced in each HPBr cartridge and in runs 2 and 3. HPBr #3 appeared to be superior in MAb production compared with HPBr #1 and #2. A similar conclusion was drawn when the HPBr was also compared with BR110 bioreactor in run 2 and run 3. This suggests that the increase of oxygenator fibers in HPBr can significantly enhance cell growth and MAb production due to the increased oxygen supply. Also the ratio of LPR to GUR was calculated for HPBr #3 and found to be significantly lower than other bioreactors. This signifies more efficient utilization of glucose in the presence of increased oxygen in the ECS.

Figure 16 compares the lactate/glucose ratio for various bioreactors. The lowest ratio was shown by HPBr devices, and these low values remain below that of the conventional bioreactors and oxygenation

methods for prolonged cell culture runs.  Ideally,
the ratio should be as close to zero as possible
throughout the cell culture run, signifying optimal
metabolic utilization of glucose.

   Table 2 is a comparison of bioreactor and
oxygenator properties.  It also contains the relative
antibody productivity of bioreactor/oxygenator
combination as a function of their physical
properties.  In this embodiment, as the number (or
surface area) of oxygenator fibers increase, and the
ratio of oxygenator to media fiber surface areas
increase, bioreactor productivity also increases.

TABLE 2

Comparative Bioreactor and Oxygenator Properties

| Property | BRR110 (Bioreactor) | OXY-1 (Oxygena-tor) | HPBr Type 1 Media | HPBr Type 1 Oxygenator | HPBr Type II Media | HPBr Type II Oxygenator | HPBr Type III Media | HPBr Type III Oxygenator |
|---|---|---|---|---|---|---|---|---|
| Material | Cellulose | Poly-ethylene | Cellulose | Poly-propylene | Cellulose | Poly-propylene | Cellulose | Poly-propylene |
| MWC or Pore Size | 10 kD | 0.2 um | 10 kD | 0.2 um | 10 kD | 0.2 um | 10 kD | 0.2 um |
| Surface Area (ft²) | 1.5 | 1.0 | 0.2 | 0.1 | 0.2 | 0.3 | 0.2 | 0.5 |
| Number of Fibers | 3150 | 1684 | 450 | 180 | 450 | 540 | 450 | 900* |
| Permeability of Media Fibers (mL/hr, mmHg) | 0.2 | n/a | $2.8 \times 10^{-2}$ | n/a | $2.8 \times 10^{-2}$ | n/a | $2.8 \times 10^{-2}$ | n/a |
| Typical IgG1 Productivity (mg) for 3C11 Hybridoma after 14 days of culture (batch run) | 23 | | 32,22 | | 68 | | Data not available at this time. | |

*Note:   Maximum number of oxygenator fibers possible in this embodiment of HPBr is about
         2000 (i.e., 1.1 ft²).  This assumes a packing density of 70%, which is in the range
         that is typical of conventional hollow fiber devices in general.

Figure 17 illustrates the level of pH control
achievable with the HPBr.  The particular data
depicted is for HPBr #3.  pH control regulates
biomass production vs. MAb production.  Lower pH,
e.g., 7.0, tends to encourage cells to remain in the
exponential growth phase to produce high biomass.
Higher pH, e.g., 7.4, tends to encourage cells to
remain in the steady state phase and therefore
exhibit higher productivity.

CLAIMS:

    1.  A hollow fiber bioreactor comprising
    a housing

      said housing having an input end, an output
end, a first port adjacent said input end and a
second port adjacent said output end;

      a first, interior bundle of porous hollow
fibers for supplying nutrient media within said
housing;

      a second, exterior bundle of hollow fibers
for providing oxygen containing gas within said
housing;

        said first and second bundles of hollow
    fibers being concentric;

        the exterior surface of said second
    bundle of hollow fibers being spaced from
    the interior wall of said housing;

    an input header for the input end of said
housing

        said input header having a first port
    for the introduction of nutrient media into
    said first, interior bundle of hollow fibers
    and a separate second port for the
    introduction of oxygen into said second
    exterior bundle of hollow fibers; and

    an output header for the output end of said
housing;

        said output header having a first port
    for the removal of media from said first
    interior bundle of hollow fibers and a
    second port for the removal of oxygen
    containing gas from said second exterior
    bundle of hollow fibers.

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2.  A hollow fiber bioreactor as defined by
claim 1 in which said first, interior bundle of
porous hollow fibers have a molecular weight cut-off
of from 1 kD to 1,000 kD and a pore size of from 0.01
$\mu$m to 5.0 $\mu$m.

3.  A hollow fiber bioreactor as defined by
claim 1 or claim 2 in which said first, interior
bundle of hollow fibers comprises cellulose,
polyethylene, polypropylene, polysulfone, polymethyl
methacrylate or polyacrylonitrile fibers.

4.  A hollow fiber bioreactor as defined by
claim 1 in which said second exterior bundle of
hollow fibers are porous hydrophobic fibers
comprising polyethylene, polypropylene or
polytetrafluoroethylene having a pore size of
from 0.01 to 0.5 $\mu$m.

5.  A hollow fiber bioreactor as defined by
claim 1 in which said second exterior bundle of
hollow fibers comprises non-porous, gas permeable
silicone or silicone copolymer fibers.

6.  A hollow fiber bioreactor as defined by
claim 1 in which the ratio of the surface area of
said second exterior bundle of hollow fibers to the
surface area of said first interior bundle of hollow
fibers is from 0.03 to 5.5.

7.  A hollow fiber bioreactor as defined by
claim 6 wherein cellular matrix or synthetic
molecules containing amino acid sequence
arginine-glycine-aspartic acid coat the available
surfaces within the housing.

8.  A hollow fiber bioreactor as defined by
claim 6 in which a combination of carbon dioxide and
an oxygenated gas is used to control pH and oxygen
levels in the extracapillary space.

9.  A hollow fiber bioreactor as defined by
claim 6 including oxygen carriers in the
extracapillary space or in the intracapillary space.

10.  A hollow fiber bioreactor as defined by
claim 6 in which hemoglobin or perfluoro chemical
emulsions are present in the extracapillary space or
in the intracapillary space.

11.  A hollow fiber bioreactor as defined by
claim 6 for the culture of insect cells.

12.  A hollow fiber bioreactor as defined by
claim 1 further comprising microcarriers for
anchorage dependent cells in said space between the
exterior surface of said second bundle of hollow
fibers and the exterior wall of said housing.

13.  A hollow fiber bioreactor as defined by
claim 12 in which stromal cells are immobilized in or
on said microcarriers for co-culture with
hematopoietic stem cells.

14.  A bioreactor comprising a housing
        an annular bundle of gas permeable hollow
    fibers within said housing;
        a lumen passing through said annular bundle
    of hollow fibers;
        means for passing an oxygen containing gas
    through said hollow fibers of said annular bundle;
        means for passing nutrient media through
    said lumen passing through said annular bundle of
    hollow fibers.

15.  A bioreactor as defined by claim 14 further
comprising a porous tube positioned within said lumen
passing through said annular bundle of hollow fibers.

16.  A bioreactor as defined by claim 14 further
comprising a bundle of porous hollow fibers
positioned within said lumen to supply nutrient media
within said housing.

17.   A method for the _ex_ _vivo_ expansion of stem
cells which comprises
        providing a hollow fiber bioreactor having
    extracapillary space
            said extracapillary space containing
        microcarriers having stromal cells
        immobilized thereon, and
        concurrently culturing stem cells and said
    stromal cells in said bioreactor.
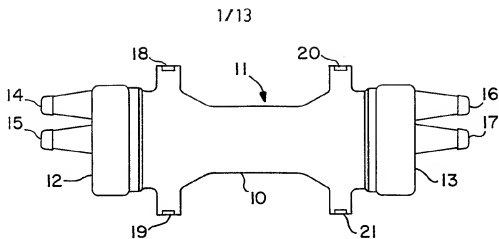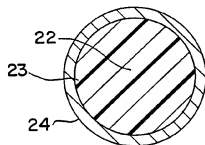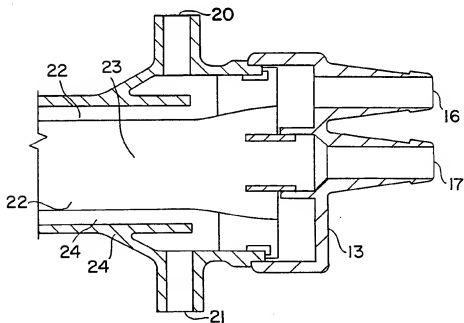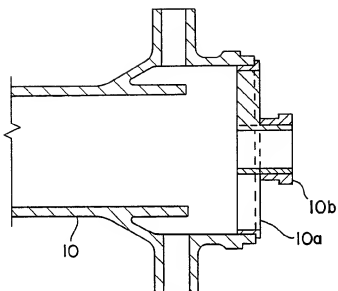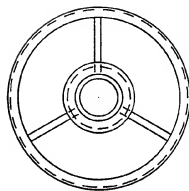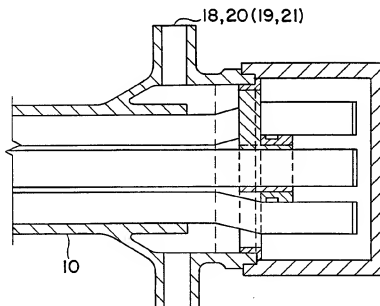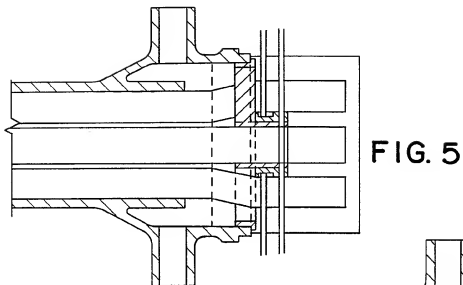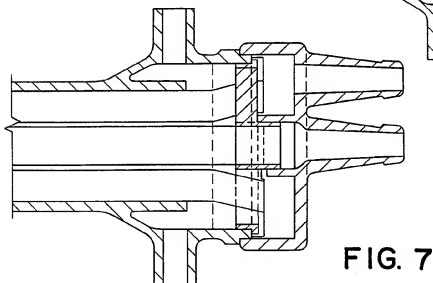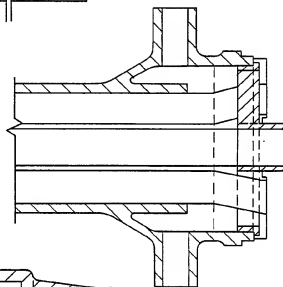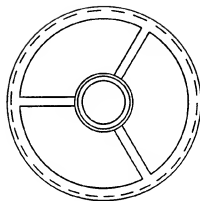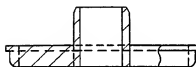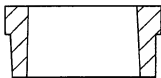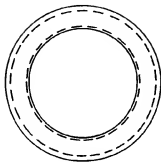
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FIG. I



FIG. 2A



FIG. 2

FIG. 3

FIG. 3A



FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8



FIG. 8A

FIG. 9



FIG. 9A

FIG. 10



FIG. 10A

FIG. II

FIG. 12

FIG. 13

FIG. 14

FIG. 15
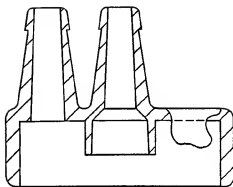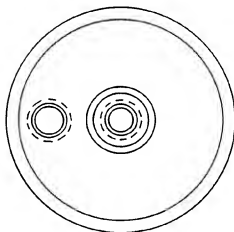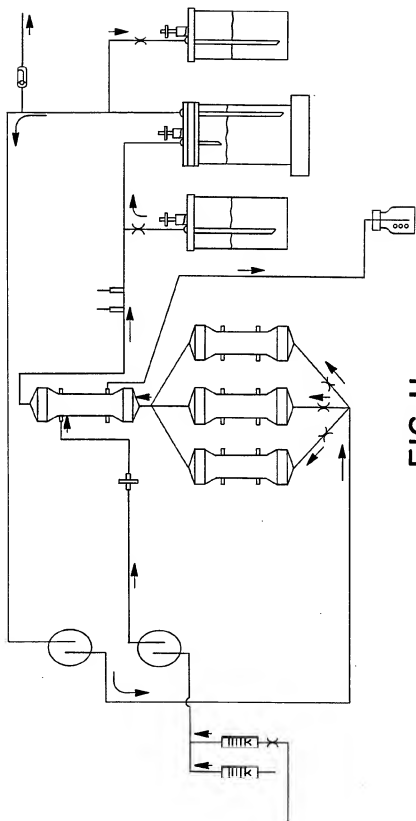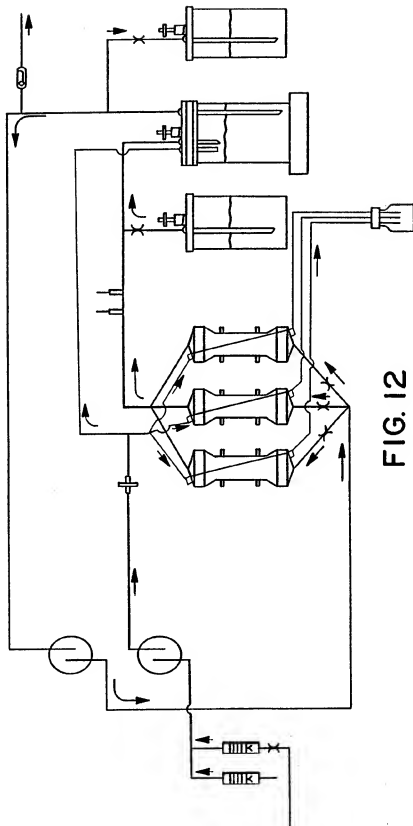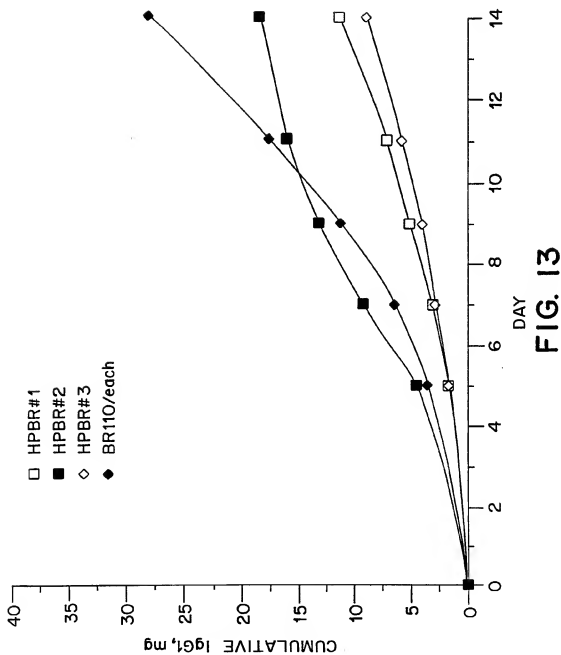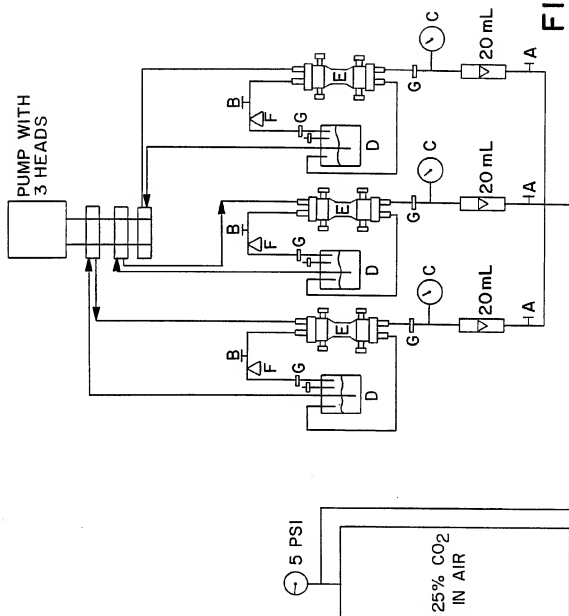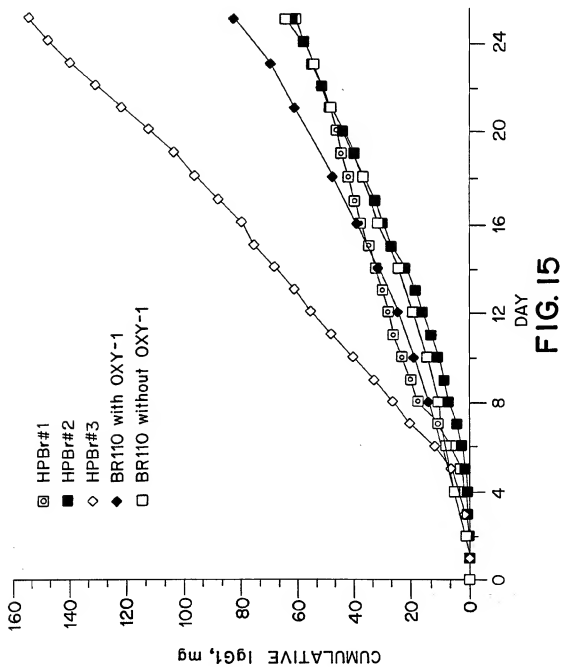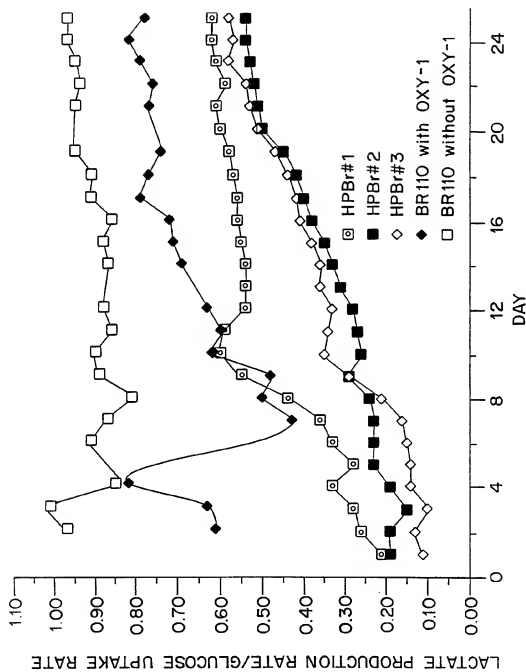
FIG. 16
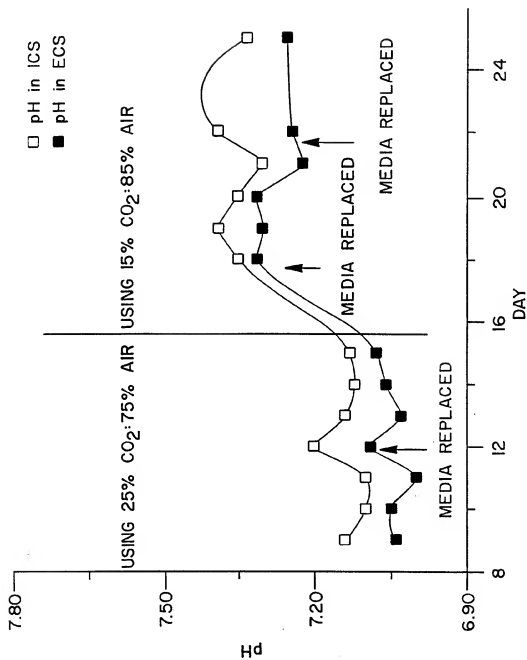
FIG. 17

# INTERNATIONAL SEARCH REPORT

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5)  :C12N 5/06; C12M 3/06
US CL  :435/240.23, 284

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S.  :  435/240.23, 240.241, 240.242, 283-286, 311, 422/48; 210/321.64, 321.78, 321.79, 321.80

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US, A, 4,220,725 (KNAZEK ET AL) 02 September 1980, see entire document. | 1-17 |
| Y | US, A, 4,720,462 (ROSENSON) 19 January 1988, see entire document. | 1-17 |
| Y | WO, A, 86/06094 (BERSILLON) 23 October 1986, see entire document. | 1-17 |
| Y | US, A, 5,278,063 (HUBBELL ET AL) 11 January 1994, see entire document. | 7 |
| Y | US, A, 4,061,736 (MORRIS ET AL) 06 December 1977, see column 15, lines 11-20. | 9,10 |
| Y | WO, A, 92/11355 (EMERSON ET AL) 09 July 1992, see entire document. | 13,17 |

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

| * | Special categories of cited documents: |
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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 JULY 1994 | 0 9 AUG 1994 |

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|---|---|
| Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | WILLIAM H. BEISNER |
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